Regulation of endodermal differentiation of human embryonic stem cells through integrin-ECM interactions.

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Public Summary:
Human pluripotent stem cells have the unique capability to generate all mature cell types, thereby offering a potentially unlimited supply of cells suitable for cell transplantation, drug screening and disease modeling. However, methods for generating specific cell populations are limited and inefficient. To address this bottle neck, we applied a novel screening approach to the generation of cells of the endodermal lineage. The endodermal lineage gives rise to many internal organs, including the pancreas, liver and intestine. We showed that human pluripotent stem cells can be encouraged to enter this endodermal lineage simply by altering the external components with which the cells interact. Cells grown on a defined matrix comprised of the extracellular molecules Fibronectin and Vitronectin efficiently differentiated into endoderm and its derivatives. In addition, we showed that the proteins that anchor cells to Fibronectin and Vitronectin are required for the generation of endodermal cell types. Together, these results provide novel protocols to specifically generate cells with endodermal properties and will aid in the development of methods to obtain mature cell types suitable for transplantation, such as pancreatic beta cells and hepatocytes. In addition, by using a high throughput cellular microarray screening platform we demonstrated that manipulation of the extracellular matrix is critical for promoting the differentiation into specific cell populations, such as endoderm.

Scientific Abstract:
Many cellular responses during development are regulated by interactions between integrin receptors and extracellular matrix proteins (ECMPs). Although the majority of recent studies in human embryonic stem cell (hESC) differentiation have focused on the role of growth factors, such as FGF, TGFbeta, and WNT, relatively little is known about the role of ECMP-integrin signaling in this process. Moreover, current strategies to direct hESC differentiation into various lineages are inefficient and have yet to produce functionally mature cells in vitro. This suggests that additional factors, such as ECMPs, are required for the efficient differentiation of hESCs. Using a high-throughput multifactorial cellular array technology, we investigated the effect of hundreds of ECMP combinations and concentrations on differentiation of several hPSC lines to definitive endoderm (DE), an early embryonic cell population fated to give rise to internal organs such as the lung, liver, pancreas, stomach, and intestine. From this screen we identified fibronectin (FN) and vitronectin (VTN) as ECMP components that promoted DE differentiation. Analysis of integrin expression revealed that differentiation toward DE led to an increase in FN-binding integrin alpha5 (ITGA5) and VTN-binding integrin alphaV (ITGAV). Conditional short hairpin RNA-mediated knockdown of ITGA5 and ITGAV disrupted hESC differentiation toward DE. Finally, fluorescence-based cell sorting for ITGA5 and ITGAV significantly enriched cells with gene expression signatures associated with DE, demonstrating that these cell surface proteins permit isolation and enrichment of DE from hESCs. These data provide evidence that FN and VTN promote endoderm differentiation of hESCs through interaction with ITGA5 and ITGAV, and that ECMP-integrin interactions are required for hESC differentiation into functionally mature cells.Cell Death and Differentiation advance online publication, 16 November 2012; doi:10.1038/cdd.2012.138.

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